In-silico Analysis of Effects Of Black Pepper Extract on Pulm Pox Virus as Biopestisides

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Abstract

Black pepper (Piper nigrum L.) is the most important spice traded internationally and is cultivated in many tropical regions of the world like India, Brazil, Vietnam, Indonesia, Malaysia and Sri Lanka. A variety of trees species are used as live stakes for supporting black pepper. However, not all are considered ideal. In ancient India spices were mixed along with different solvent and used as medicine to treat different diseases. This spice plants are having extraordinary chemical which we will find as fragrance, taste . We have used these properties of these spice plants and spices to get some targeted medicine for different diseases. We have taken Black Pepper for the targeted therapy for the Pulm Pox Virus, which is a common disease in the topical region of the country.

KEY WORDS: BLACK PEPPER, MOLECULAR DOCKING, PHARMACOPHORE, PULM POX VIRUS

INTRODUCTION

Black Pepper is basically produced in southeast and south Asia and has been known to India cooking since at least 2000 BCE. *Piper nigrum L*. (*P. nigrum;* black pepper) belonging to the family Piperaceae is considered the king of spices due to its spicy savor (Abbasi et al., 2010; Ahmad et al., 2012a, 2013). *P. nigrum* is cultivated throughout the world and is native to tropical and subtropical regions of India (Ahmad et al., 2010, 2012a). High pungency in black pepper fruits indicated the presence of piperine. Piperine, the most active component in the fruits of *P. nigrum* L., denotes the quality and value of spiciness (Ahmad et al., 2011a; Bhat et al., 1995; Philip et al., 1992). Peppercorn from *P. nigrum* can be used in food processing, as crude drugs, and can also be used as food additives (Srinivasan, 2007). Regarding medicinal applications, *P. nigrum* has pronounced antibacterial, antifungal, antiviral, antimutagenic, and antioxidant activities (Abbasi et al., 2010; Ahmad et al., 2010, 2012a; Dorman and Deans, 2000; Saxena et al., 2007). Nerolidol and b-caryophyllene isolated from *P. nigrum* have anesthetic activity (Santra-Mantra et al., 2005). The fruits of this species are also used to treat respiratory tract diseases and infections such as Pulm Pox Virus, cold extremities and sore throat, digestive problems including chronic indigestion, colon toxins, colic, and diarrhea, and fevers including congestion fever and intermittent fever and also control obesity (Ahmad et al., 2011a; Ravindran, 2000). *P. nigrum* also claimed to possess antiapoptotic activity, antidepressant, analgesic, antiinflammatory, antimetastatic, antispasmodic, antispermatogenesis, antithyroid, hepatoprotective, insecticidal, larvicidal, and pesticidal activities (Balkrishna, 1995; Kumar et al., 2007; Li et al., 2007; Mishra and Singh, 2009; Pathak and Khandlewal, 2006; Scott et al., 2008). Plants produced a variety of active metabolites that act as a defense system against various pathogenic agents (Ahmad et al., 2011b, 2012b, 2012c). Black pepper photochemical name is Pelerine .basically useful for health problems in home. Black pepper properties include for the bioactive and preservative. It uses for spices Worldwide. It is good for improve metabolic rate and inhibits adipose cell growth. It helps for also digestion due to the pelerine which stimulate the gastric fluids. The pipeline present in black pepper helps body to absorb some of the nutrients found in certain foods. Pipeline treatment has also been evidenced to lower lipid peroxidation in vivo and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes in a number of experimental situations of oxidative stress. The chemicals of piperine is C17H19NO3 .

MATERIALS and METHODS

Various pharmacophores of Black Pepper leaves have been listed and their respective SDF were taken accordingly from Pubchem, Molinstincts, and Chebi. The enzyme corresponding to microbe of Asthama has been taken from BRENDA (Braunschweig Enzyme Database). Then, the PDB (Protein Data Bank) code was found from RCSB (Research Collaboratory for Structural Bioinformatics). The above mentioned information was then processed in Discovery Studio to initiate Docking. The following screenshots are taken from Discovery Studio, showing positive results of docking;

Table 1: The list of pharmacophores and the targeted genes

Sl.No	Black Pepper Pharmacophores	Targeted Plant Disease Causing Microbial (Pulm pox virus) Gene	PDB No of the Genes
1	Beta-pinene	polyprotein	6QZU
2	Limonene	PiPO	6KZW
3	P- cymene	PIPO	5UVR
4	Piperazine		

Protein identification and preparation

The reported molecular targets responsible for Gene are taken (Table 1) and the X-ray crystallographic structures of these target proteins were retrieved from protein data bank (PDB). The retrieved PDB structures contain water molecules, heavy atoms, cofactors, metal ions etc. and these structures do not have information about topologies, bond orders and formal atomic charges. Hence the downloaded PDB structures were prepared using 'prepare protein' protocol of Discovery Studio 4.0. The target proteins were prepared by removing all water molecules, ligands and other hetero atoms from the structures. Hydrogen atoms were added to the atoms to satisfy their valencies. The structures were then energy minimized by applying CHARM force field to remove the steric clashes between the atoms in order to get stable conformation.

Active site identification

The binding sites of the receptor proteins were predicted based on 'receptor cavity method' using Accelry's Discovery Studio 4.0. Using this protocol, active sites of the target receptor were identified based upon the inhibitory property of the amino acid residues present in the binding sites.

Ligand preparation and filtration

A collection of 5 phytocompounds from Black Pepper were taken as ligands for docking analysis. The 3D structures of these compounds were downloaded from PubChem database. These ligands were then cleaned up, calculated 3D coordinates and generated ligand conformations by applying 'prepare ligand protocol' of Discovery Studio 4.0. After preparation, the compounds were filtered based on the molecular properties for predicting their solubility and permeability in drug discovery. The best known of the physical property filters is Lipinski's "rule-offive", which focuses on bioavailability. The rule states that the compounds have molecular mass less than 500 daltons, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and an octanol-water partition coefficient log P not greater than 5 **(Lipinski et al.,2001).** The filtered compounds were then used for docking analysis.

Docking

The anti-inflammatory activity of all the 4 phytochemicals reported from Black Pepper was assessed by docking these compounds against the respective active sites of the target proteins. Discovery studio 4.0 was used in this study to find the interacting compounds of Black Pepper with the selected targets of arthritis. Strategies of Discovery Studio 4.0 are to exhaustively dock or score possible positions of each ligand in the binding site of the proteins. Docking study of the target proteins was done with natural compounds derived from Black Pepper to find the preferred orientation and binding affinity of the compounds with each target protein using scoring functions. A molecular dynamics (MD) simulated-annealing-based algorithm, namely, CDOCKER was used to score the interacting compounds. This method uses a gridbased representation of the protein-ligand potential interactions to calculate the binding affinity **(Wu et al., 2003).** CDOCKER uses soft-core potentials, which are found to be effective in the generation of several random conformations of small organics and macromolecules inside the active site of the target protein. Ligands were docked to the proteins followed by scoring them for their relative strength of interaction to identify candidates for drug development. The final poses were then scored based on the total docking energy, which is composed of intramolecular energy of ligand and the ligand-protein interaction. The lowest energy structure was taken as the best fit. Interpretation of the values was done using standards provided by Discovery Studio such as CDOCKER energy, CDOCKER interaction energy, hydrogen bonds, binding energy etc.

Drug likeliness

Drug-likeness is a qualitative concept used in drug design to evaluate how the substance acts like drug with respect to factors like bioavailability. The molecular properties which influence absorption, distribution, metabolism, excretion and toxicity are recognized as a long side therapeutic potency as

key determinants of whether a molecule can be successfully developed as a drug **(Zhang et al., 2012).** These parameters are responsible for about 60 percent failures of all drugs in the clinical phases and so the prediction of ADMET properties plays a significant role in new drug discovery process (**Hire et al., 2012)**. Thus, it has become imperative to design lead compounds which would be easily Gastricly absorbed, easily transported to their targeted site of action, not easily converted into toxic metabolic products and easily eliminated from the body before accumulating in sufficient amounts. The ADMET properties of the compounds were analyzed for drug like candidates.

RESULT AND DISCUSSION

Protein preparation and active site identification

The three dimensional structures of the identified target proteins were retrieved from the protein data bank. PDB ID of the targeted protein structure are mentioned in Table 1.

Ramachandan Plot of the targeted gene

The Ramachandran plot is among the most central concepts in structural biology, seen in publications and textbooks alike. However, with the increasing numbers of known proteinstructures and greater accuracy of ultra-high resolution protein structures, we are still learning more about the basic principles of protein structure. The use of torsion angles to describe polypeptide and protein conformation was developed by Sasisekharan as part of his studies of the structure of collagen chains during his work as a graduate student in the research group of G.N. Ramachandran. The power of this approach was readily apparent and its use quickly became widespread. Using revised definitions, this so-called Ramachandran plot or ϕ, ψ-plot has remained nearly unchanged in the ensuing fifty years and continues to be an integral tool for protein structure research and education.

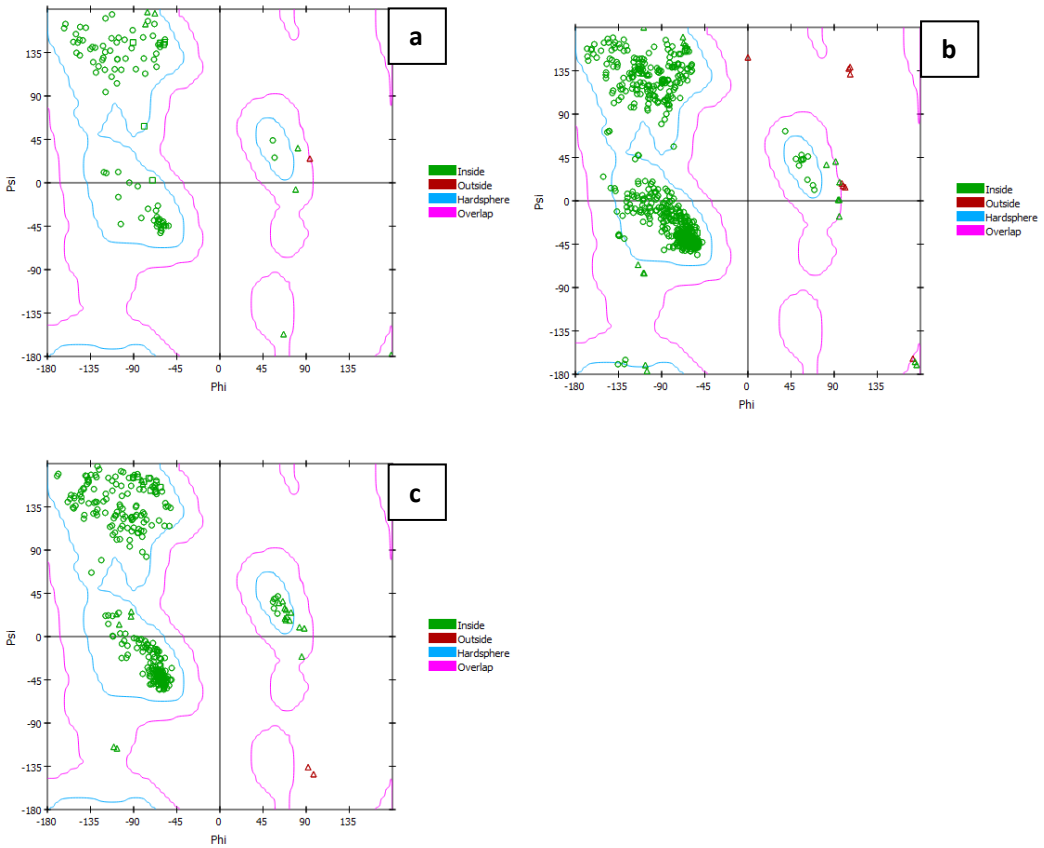


Fig 1: Ramachandan Plot of (a) 5UVR, (b) 6KZW, (c) 6KZU

Hydrophobicity Plot of the Genes:

Protein–protein interactions (protein functionalities) are mediated by water, which compacts individual proteins and promotes close and temporarily stable large-area protein–protein interfaces. In their classic article, Kyte and Doolittle (KD) concluded that the "simplicity and graphic nature of hydrophobicity scales make them very useful tools for the evaluation of protein structures." In practice, however, attempts to develop hydrophobicity scales (for example, compatible with classical force fields (CFF) in calculating the energetics of protein folding) have encountered many difficulties

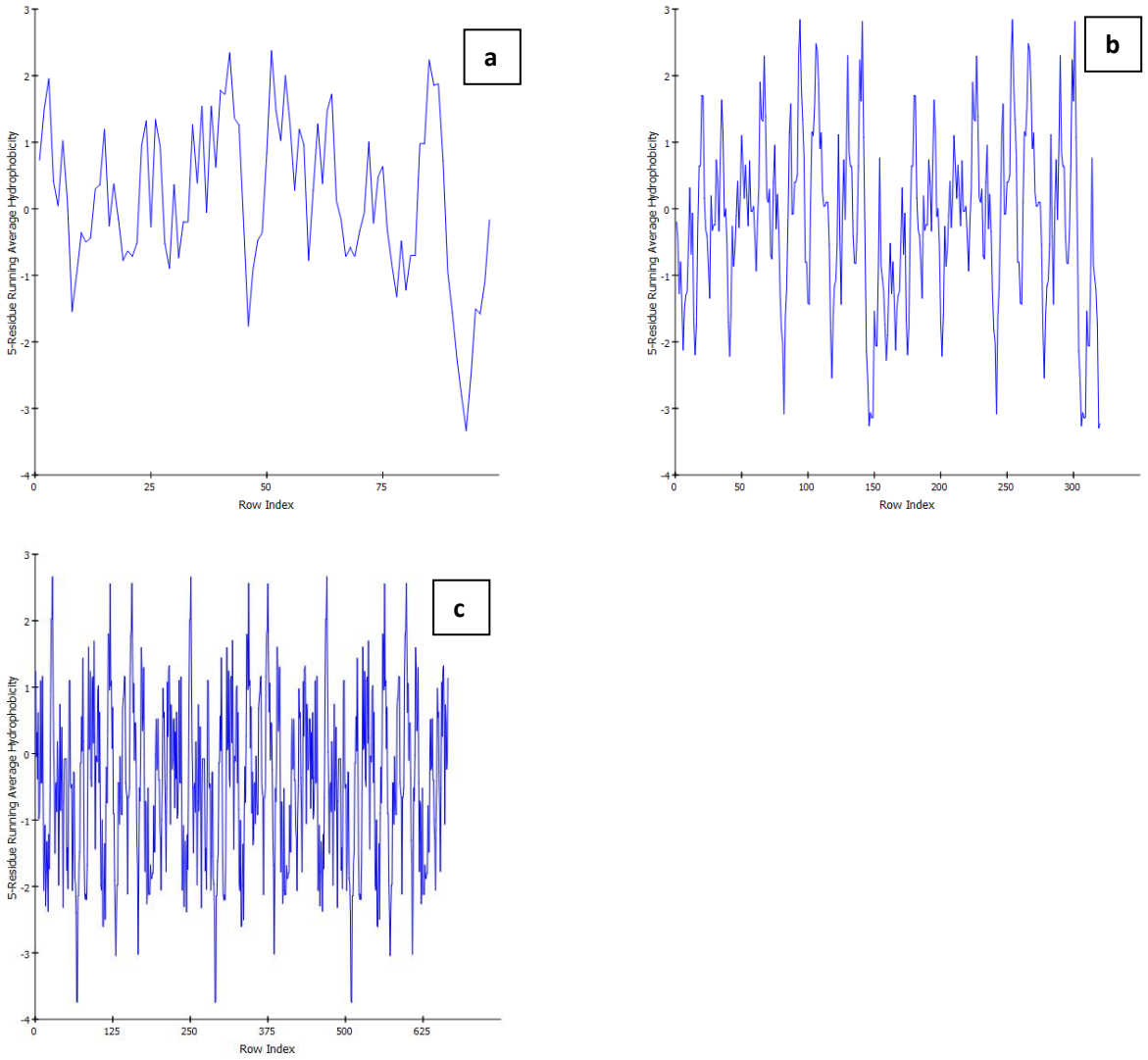


Fig 2: Hydrophobicity Plot of (a) 5UVR, (b) 6KZW, (c) 6KZU

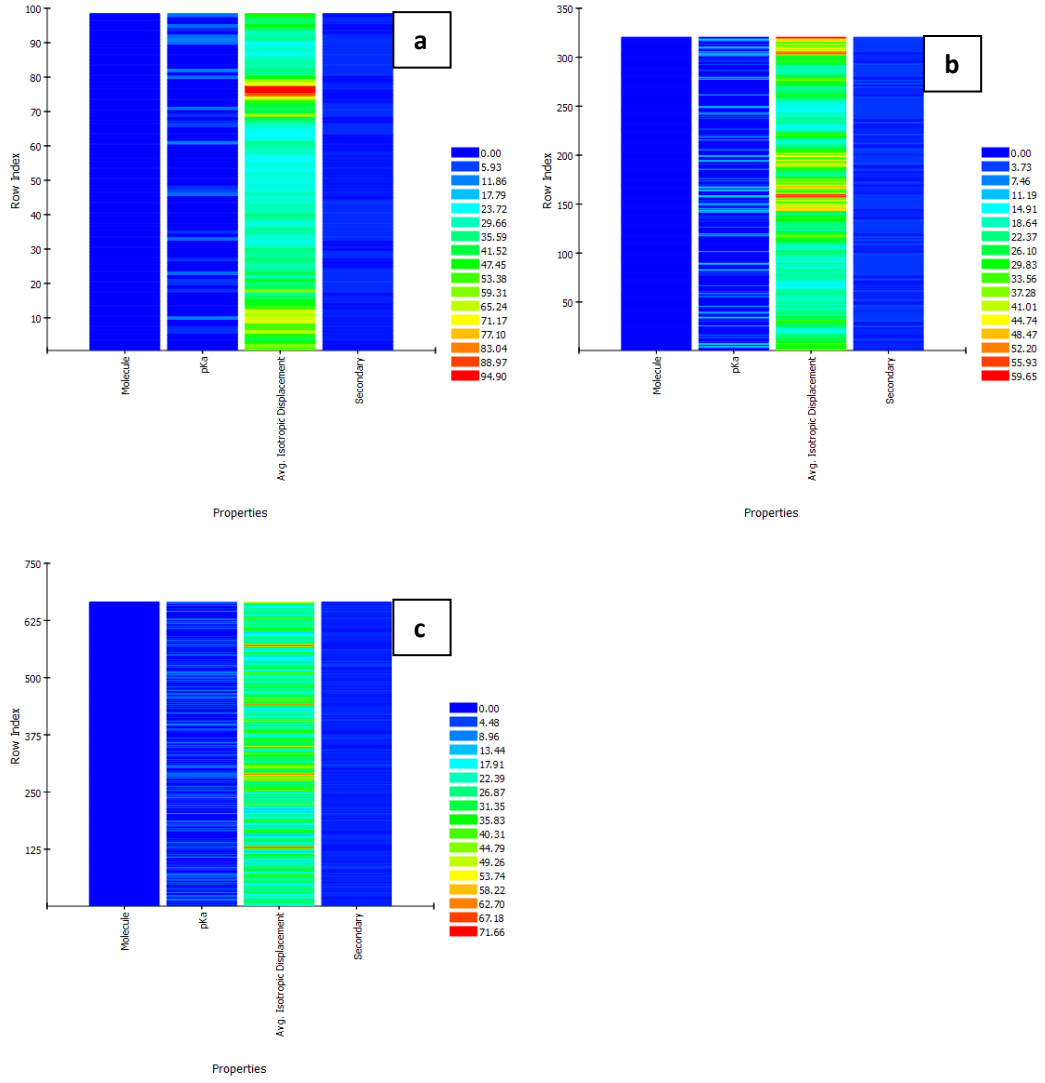
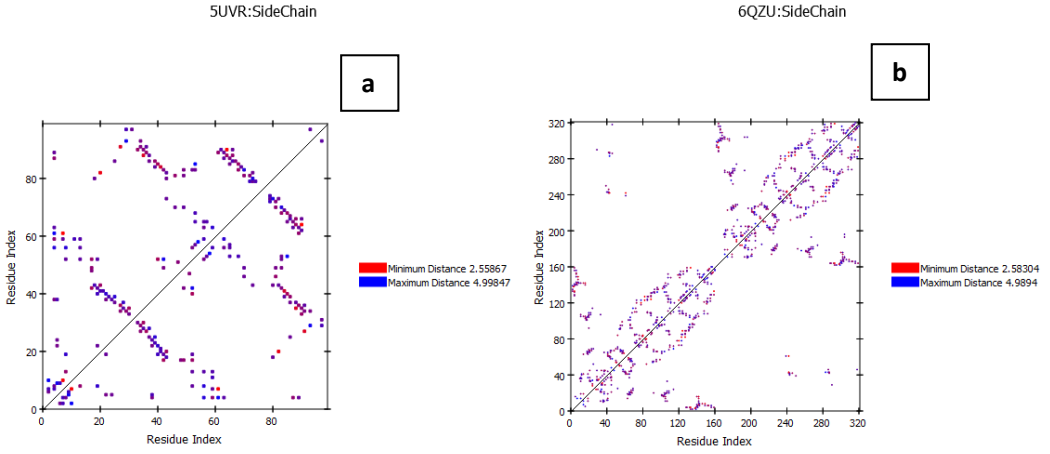


Fig 3: Heatmap of (a) 5UVR, (b) 6KZW, (c) 6KZU



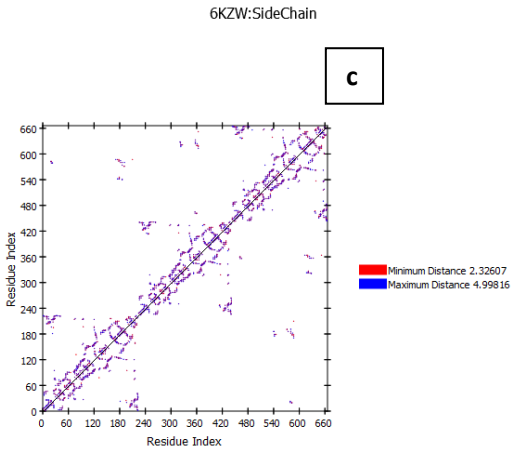
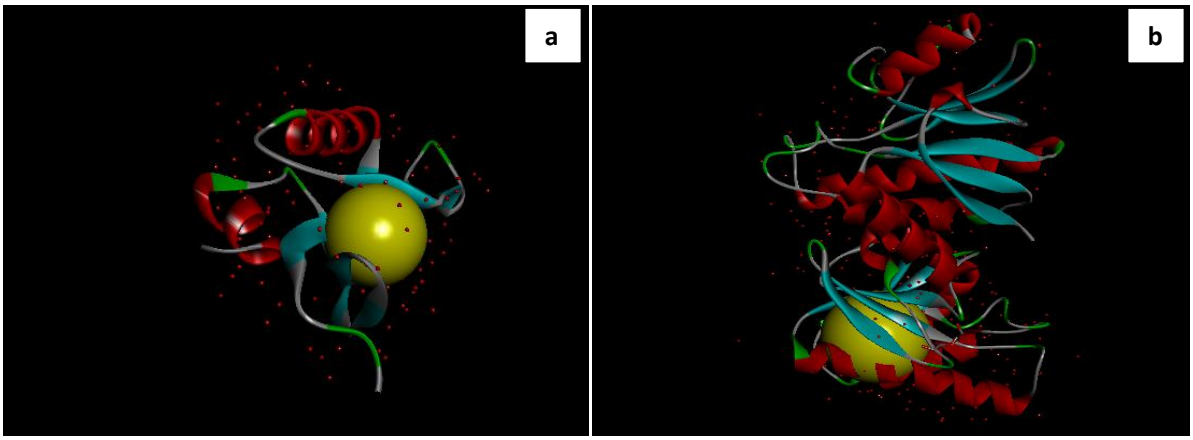


Fig 4: Side chain Analysis Plot of (a) 5UVR, (b) 6KZW, (c) 6KZU

Ligand preparation

4 of the pharmacophores are satisfied Lipinski rule and are expected to be active compounds after Gastric administration. The ligand molecules with least binding energy are considered as compounds with highest binding affinity. This binding affinity indicated a focused interaction between the above compounds with the targets compared to others. The parameters for finding the best inhibitors such as CDOCKER energy, CDOCKER interaction energy and number of hydrogen bonds were also evaluated. CDOCKER energy is the combined energy produced by the sum of internal ligand strain energy and receptor-ligand interaction energy where, CDOCKER interaction energy is the interaction energy between the protein and ligand and the values of these two parameters indicate the strength of interaction between the proteins and the ligands. Besides least binding energy, compounds with least atomic energy difference between CDOCKER energy and CDOCKER interaction energy were analyzed. Based on CDOCKER energy and CDOCKER interaction energy, Fig 5 is showing the result.



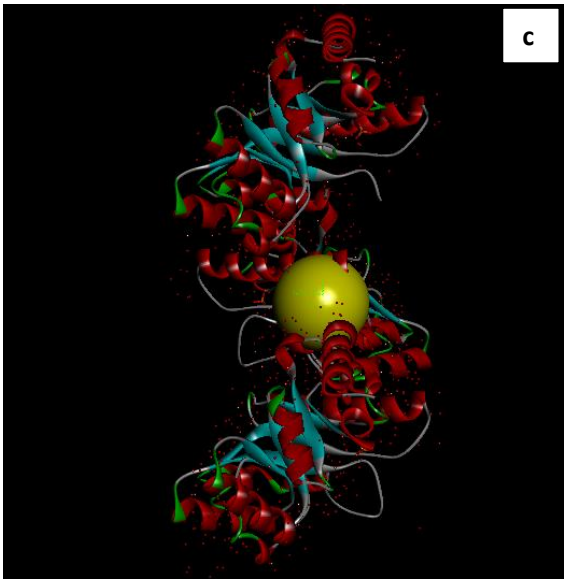
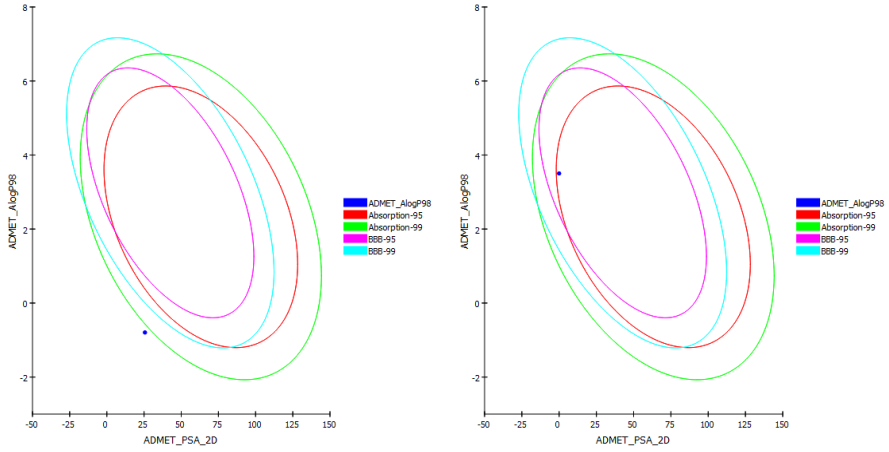


Fig 5: Docking Aaalysis of (a) 5UVR, (b) 6KZW, (c) 6KZU

ADMET Evaluation

Considering the comparable CDOCKER energy, interaction energy and binding energy, three compounds were forwarded for ADMET analysis. These studies are based on the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the compounds. These properties provide insights in to the pharmacokinetic properties of the compounds and were checked using Discovery Studio's built in ADMET protocol. The various parameters tested in this study were aqueous solubility, Blood Brain Barrier (BBB) level, Hepatotoxicity, Absorption level, AlogP and CYPD26. Pharmacokinetic properties of the best fit phytochemicals showed that two of the compounds had passed all the pharamacokinetic parameters. The compounds that passed the parameters were N-methyltyramine and dalbergioidin. These compounds were thus selected as the best compounds in this study as they had good interaction scores along with ADMET properties.



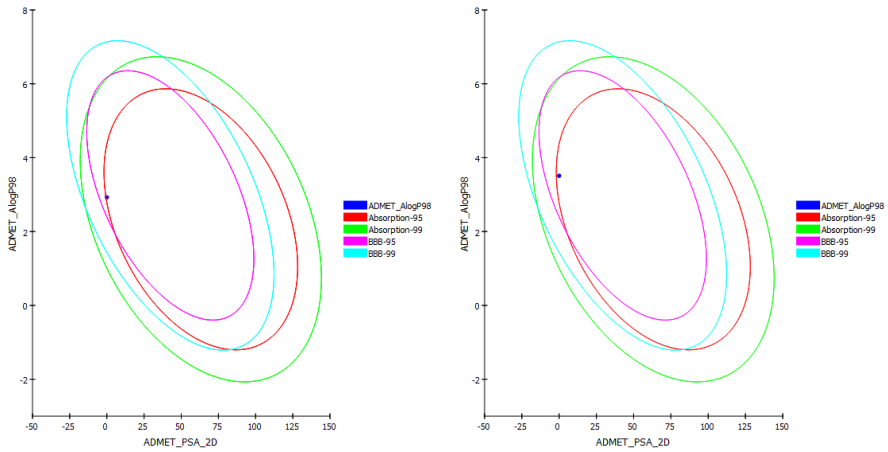


Fig 6: ADMET analysis Report of the Pharmacophores of the Pepper

CONCLUSION

The identified pharmacophores can be isolated from the Black Pepper and can be commercialized as the natural drug for the Pulm Pox Virus Gene which is having lesser harmful side effect from the chemotherapeutic drug available in the market. This drug will also be very cheaper from the available drugs and these drugs are also not harmful for the normal cells as they are derived from the natural products.

The unique feature of the study is to targeted gene therapy for a particular cancer. This will help our future medicine to be completely allied to the Pharmachophores and the uses of synthetic and carcinogenic drug will reduce.

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